[18F]fluoro-deoxyglucose (FDG) in single cells, which was found consistent with fluorescence imaging of a glucose analog. We also verified that dynamic uptake of FDG in single cells followed the standard two-tissue compartmental model. A difference in FDG uptake rates using single-cell analysis versus bulk cell analysis revealed that radioluminescence microscopy is an important tool to investigate single-cell biology. Biological studies at the single-cell level aim at elucidating the effect of the cell cycle on the FDG uptake in breast cancer cell populations. These experiments can also find metabolic differences to determine subpopulations in clonal cell populations.

2026-Pos Board B756
Quantitative Storm using Organic Dyes
Veronica Pessino[1], Wei Qiang Ong[2], Bo Huang[3].
[1]Biophysics, UCSF, San Francisco, CA, USA, [2]Pharmaceutical Chemistry, UCSF, San Francisco, CA, USA, [3]Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA, USA.
Quantitative super-resolution imaging is important for understanding and determining the exact stoichiometry of molecular complexes or oligomerization states of proteins within a cell. This has been done previously using fluorescence proteins, however these proteins require extensive computational correction due to their blinking. Organic dyes are commonly used for super-resolution imaging because of their high photon numbers, unfortunately blinking corrections for these dyes are very difficult. Here, we develop a technique for quantitative super resolution microscopy that allows us to count the absolute number of molecules using organic ''caged'' dyes, which do not blink. We calibrate our system and find that caged-fluorescein and caged-carborhodamine, when conjugated with SNAP-tag labeling, allow for quantitative counting of targets. We apply this technique to the characterization of the oligomerization states of chemokine receptors in mammalian cells.

2027-Pos Board B757
Mn^{2+} Induced Triplet Blinking and Photobleaching of Single Molecule Cyanine Dyes
Monika A. Ciuba, Elana M.S. Stennett, Marcia Levitus.
Arizona State University, Tempe, AZ, USA.
Organic fluorescent probes are used for a myriad of applications including imaging and the study of dynamics and kinetics of biological macromolecules. However, they are susceptible to blinking and bleaching, which complicate data analysis and limit observation time of single molecule experiments. It is thought that transitions into triplet states, and formation of other dark states from the triplet, result in blinking, and are the first step in photobleaching reactions. Recently, however, dark states have proved useful with the emergence of super resolution imaging techniques, which highlight the importance of understanding the nature of blinking and bleaching. Previously, we reported on our observation that TAMRA dye conjugated to DNA experiences an increased intersystem crossing rate when in the presence of Mn^{2+}. In recent work, we investigated the effect of Mn^{2+} on commonly used cyanine dyes Cy3, Cy3B, and Cy5. As for TAMRA dye, transient absorption and fluorescence correlation spectroscopy experiments confirm the formation of the triplet state of these cyanines in Mn^{2+} buffers. The significant structural differences between the rhodamine TAMRA and the investigated cyanines suggest that triplet formation in the presence of Mn^{2+} is a universal phenomenon. Given the wide-ranging use of Mn^{2+} in biophysical studies_it is often substituted for Mg^{2+} in mechanistic studies of polymerases and restriction enzymes_and the prevalence of fluorescence-based single molecule techniques, these results underscore the importance of understanding the photophysical origin of dark states. Additionally, it may be useful to use Mn^{2+} to control the triplet state lifetime in super-resolution imaging applications.

2028-Pos Board B758
An Optimized Azimuthal Scanning Platform for TIRF and HILO Imaging
Avtar Singh, Eli A. Doris, Alex Song, Warren R. Zipfel.
Cornell University, Ithaca, NY, USA.
Due to its ability to selectively excite fluorophores near a substrate, Total Internal Reflection Fluorescence (TIRF) microscopy has gained a prominent role in biological imaging and is particularly well-suited for cell membrane studies and in vitro single molecule assays. Similarly, a highly inclined laminated optical sheet (HILO) has been used for high S/N imaging away from the glass-water interface. Although rapid azimuthal scanning of the laser beam has been shown to confer certain advantages, namely fringe reduction and better uniformity of illumination, the effects of optical design on field-of-view and TIRF quality has not been systematically studied. Using commercial ray tracing software, we demonstrate how various choices in the optical system design affect each of these parameters. Using these simulations as a guide, we have built a simple circular scanning microscope for TIRF and HILO imaging using a standard inverted microscope, an XY galvanometer scanner and inexpensive lab-built driver electronics and software. Microscope design was optimized to provide a large field of view without compromising TIRF quality and the performance is demonstrated under demanding conditions (high background fluorescence). Finally, we demonstrate that circle-scanned TIRF is successful for a wide range of applications, including live-cell studies, single molecule assays and super-resolution microscopy.

2029-Pos Board B759
Cathodoluminescence-Activated Imaging by Resonance Energy Transfer: A New Approach to Imaging Nanoscale Aqueous Biodynamics
Connor G. Bischak[1], David M. Kaz[1,2], Craig L. Hetherington[1], Jake T. Precht[1], Xavier Marti[3], James D. Clarkson[3], Carolina Adamo[4], Darrell G. Schlom[4,5], Ramamoorthy Ramesh[3,6], Shaul Aloni[6,7], D. Frank Ogletree[6,7], Naomi S. Ginsberg[1,2].
[1]Department of Chemistry, University of California Berkeley, Berkeley, CA, USA, [2]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [3]Materials Science and Engineering, University of California Berkeley, Berkeley, CA, USA, [4]Materials Science and Engineering, Cornell University, Ithaca, NY, USA, [5]Kavli Institute at Cornell for Nanoscale Science, Cornell University, Ithaca, NY, USA, [6]Materials Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [7]Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
The organization, complexing, and aggregation of biomolecules in solution and in lipid membranes lie at the foundation of many critical processes in metabolism, signaling, and disease. Yet, our understanding of these phenomena remains limited by our ability to probe small aqueous volumes under physiological conditions. Here we present progress toward a high-brightness, rapidly scannable, nanoscale light source aimed at interrogating biomolecular dynamics in highly concentrated aqueous environments below the diffraction limit. This light source consists of a thin film of cathodoluminescent (CL) material excited by a low energy, tightly focused electron beam from a scanning electron microscope (SEM). By integrating the CL film into a liquid sample cell, a biological sample can be isolated from the SEM's vacuum environment. Our CL optical source consists of a highly crystalline film of cerium-doped yttrium aluminum perovskite (YAlO3:Ce3+), an efficient CL material. With our custom CL detection apparatus, we have shown that the CL excitation volume in the film is at least as small as 20 nm in diameter. Because the CL film is only 10 nm thick, optical excitations from the $Ce3^{+}$ dopants in the CL film can be non-radiatively transferred to adjacent fluorescently labeled molecules in an encapsulated sample volume via Förster resonance energy transfer (FRET). We characterize this non-invasive interaction by coupling the donor CL film to various acceptor materials. When imaging biological systems, we anticipate achieving a spectrally-specific scanning optical microscopy with at least 20 nm lateral resolution and 10 nm axial resolution, with video frame rates, and no moving parts.

2030-Pos Board B760
Profiling Cancer Cell Intrinsic Fluorescence with the Spectral Camera-Phasor Analysis Method
Michelle A. Digman, Enrico Gratton, Hongtao Chen.
Biomedical Engineering, University of California, Irvine, Irvine, CA, USA.
Advances in hyper- or multi-spectral based imaging cameras have expanded new areas of cellular research for the last 20 years. Most recently, intensity signals can now be detected with a line spectrograph in which light is transmited over an imaging signal to an ultra sensitive CCD camera with fast readout speed. However, spectrally resolving these images is highly problematic given that most fluorescent emission spectra are broad and superimposed one with another. In order to spectrally resolve these images with high spectral resolution, we have developed the phasor analysis, used in the first and second harmonic, where each pixel in the image is used to construct a spectral profile that is Fourier transformed to produce the co-ordinates of the pixel in a polar plot. This graphical representation is free of fitting routines and can easily separate linear combinations of multiple spectral components. For this application we used the Andor's iXon Ultra EMCCD camera to obtain spectral emission of cancer cell autofluorescence excited with a multiphoton laser source. Using the multispectral phasor analysis we have been able to identify autofluorescence of cell membrane, mitochondria, nucleus and other organelles. Given the complexity of these biochemical species, we treated the cells with various agents used to perturb metabolic states, membrane fluidity and cellular function to identify changes in spectroscopic signals. A map of these chemical species can provide important information to identify proliferation, stress and dysplasia at the single cell level and can be applied to in the study of cancer and other diseases. Work supported in part by NIH grants P50 GM076516 and P41 GM103540.